CLAIMS: 65 102

1. A DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule.

2. A DNA sequence according to claim 1, wherein the TRAF molecule is TRAF2.

3. A DNA sequence according to claim 2, wherein said encoded protein binds to at least the 222-501 amino acid sequence of TRAF2.

4. A DNA sequence according to ~~any one of claims 1 to 3,~~ claim 1 selected from the group consisting of:

(a) a cDNA sequence of the herein designated clone 9 comprising the nucleotide sequence depicted in Fig 3a.;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4;

(c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig. 5a;

(d) a fragment of a sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

(e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

5. A DNA sequence according to ~~any one of claims 1 to 4,~~ claim 1 selected from the sequences contained in the herein designated cDNA clones 9 and 15.

6. A DNA sequence according to ~~any one of claims 1 to 4,~~ claim 1 which DNA encodes a protein that also modulates NF-κB activity.

7. A DNA sequence according to claim 6, selected from the sequences contained in the herein designated cDNA clone 10.

8. A DNA sequence according to claim 1 ~~or 6,~~ comprising the DNA sequence encoding the protein NIK (as herein defined).

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9. A DNA sequence encoding the protein NIK, isoforms, fragments or analogs thereof, said NIK, isoforms, fragments or analogs thereof being capable of binding to TRAF2 and which is capable of modulating the activity of NF-κB.

10. A DNA sequence according to claim 9, selected from the group consisting of:

a) a cDNA sequence derived from the coding region of a native NIK protein;

b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active NIK; and

c) DNA sequences which are degenerate as a result of the genetic code to the sequences defined in (a) and (b) and which encode a biologically active NIK protein.

11. A DNA sequence according to claim 9 ~~or 10~~ comprising at least part of the sequence depicted in Fig. 6 and encoding at least one active NIK protein, isoform, analog or fragment.

12. A DNA sequence according to claim 11 encoding a NIK protein, isoform, analog or fragment having at least part of the amino acid sequence depicted in Fig. 6.

13. A vector comprising a DNA sequence according to ~~any one of claims 1-12.~~ claim #55

14. A vector according to claim 13 capable of being expressed in a eukaryotic host cell.

15. A vector according to claim 13 capable of being expressed in a prokaryotic host cell.

16. Transformed eukaryotic or prokaryotic host cells containing a vector according to ~~any one of claims 13-15.~~ claim 13

17. A TRAF-binding protein, isoforms, fragments, analogs and derivatives thereof, encoded by a DNA sequence according to ~~any one of claims 1-12,~~ claim 1 said protein, isoforms, fragments, analogs and derivatives thereof being capable of binding to at least the portion of the TRAF2 protein between amino acids 222-501 of TRAF2.

18. A protein according to claim 17 being the protein encoded by herein designated clone 10.

19. A protein, isoforms, fragments, analogs and derivatives thereof, according to claim 17 being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the DNA sequence according to any one of claims 1-12.

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20. A NIK protein, isoforms, analogs, fragments and derivatives thereof according to claim 19, wherein said protein, isoforms, fragments and derivatives have at least part of the amino acid sequence depicted in Fig. 6.

21. A method for producing a protein, isoform, fragment, analog or derivative thereof ~~according to any one of claims 17-19,~~ which comprises growing a transformed host cell according to claim 16 under conditions suitable for the expression of said protein, isoform, fragment, analog or derivative thereof, effecting post-translational modification, as necessary, for obtaining said protein, isoform, fragment, analog or derivative thereof, isolating said expressed protein, isoform, fragment, analog or derivative.

22. Antibodies or active fragments or derivatives thereof, specific for ~~the TRAF-binding protein, isoform, analog, fragment or derivative thereof~~ according to claim ~~17 or 18,~~ or specific for the NIK protein, isoform, analog, fragment or derivative thereof according to claim 19 or 20.

23. A method for the modulation or mediation in cells of the activity of NF-κB or any other intracellular signaling activity modulated or mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative thereof according to ~~any one of claims 17-20~~ binds, said method comprising treating said cells by introducing into said cells one or more of said protein, isoform, analog, fragment or derivative thereof in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more protein, isoform, analog, fragment or derivative thereof in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

24. A method according to claim 23, wherein said treating of cells comprises introducing into said cells a DNA sequence encoding said ~~protein, isoform, fragment, analog or derivative~~ in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

25. A method according to claim 23 or 24 wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of:

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on

the surface of said cells to be treated and a second sequence encoding a protein selected from the said protein, isoforms, analogs, fragments and derivatives ~~according to any one of claims 17-20~~, that when expressed in said cells is capable of modulating/mediating the activity of NF-κB or any other intracellular signaling activity modulated/mediated by TRAF2 or other said molecules; and

(b) infecting said cells with said vector of (a).

26. A method for modulating TRAF2 ~~modulated/mediated effect~~ on cells comprising treating said cells with antibodies or active fragments or derivatives thereof, according to claim 22, said treating being by application of a suitable composition containing said antibodies, active fragments or derivatives thereof to said cells, wherein when the TRAF2-binding protein or portions thereof of said cells are exposed on the extracellular surface, said composition is formulated for extracellular application, and when said TRAF2-binding proteins are intracellular said composition is formulated for intracellular application.

27. A method for modulating the TRAF2 modulated/mediated effect on cells comprising treating said cells with an oligonucleotide sequence encoding an antisense sequence for at least part of the DNA sequence encoding a ~~TRAF2 binding protein~~ according to ~~any one of claims 1-11~~, said oligonucleotide sequence being capable of blocking the expression of the TRAF2-binding protein.

28. A method according to claim 27 wherein said oligonucleotide sequence is introduced to said cells via a virus of claim 25 wherein said second sequence of said virus encodes said oligonucleotide sequence.

29. A method for modulating the TRAF2 modulated/mediated effect on cells comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding a ~~TRAF2 binding protein~~ according to ~~any one of claims 17-20,~~ is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said TRAF2-binding protein in said cells.

30. A method for isolating and identifying ~~proteins~~, according to ~~any one of claims 17-20~~, capable of binding directly to TRAF2, comprising applying the yeast two-hybrid

procedure in which a sequence encoding said TRAF2 is carried by one hybrid vector and sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said TRAF2.

31. A method according to ~~any one of claims 23-30~~ wherein said protein is NIK or at least one of the NIK isoforms, analogs, fragments and derivatives thereof.

32. A pharmaceutical composition for the modulation of the TRAF2 modulated/mediated effect on cells comprising, as active ingredient at least one ~~TRAF2-binding proteins~~, according to ~~any~~ one of claims 17-20; ~~its biologically active fragments, analogs, derivatives or mixtures thereof.~~

33. A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding at least one ~~TRAF2-binding protein, isoform, active fragments or analogs~~, according to any one of ~~claims 17-20~~.

34. A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the TRAF2-binding protein mRNA sequence according to any one of ~~claims 1-11.~~

35. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a ~~protein~~ according to ~~any one of claims 17-20~~ binds, said composition comprising an effective amount of a protein encoded by clone 10 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein encoded by clone 10 with TRAF2 or any other molecule to which a protein encoded by clone 10 binds.

36. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a ~~protein~~ according to ~~any one of claims 17-20~~ binds, said composition comprising an effective amount of a NIK protein, isoform, fragment, analog or derivative thereof, or a DNA molecule coding therefor, or a molecule capable of disrupting

the interaction of said NIK protein, isoform, fragment, analog or derivative thereof with TRAF2 or any other molecule to which said NIK protein, isoform, fragment, analog or derivative binds.

37. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which the protein NIK binds, said composition comprising a molecule capable of interfering with the protein kinase activity of NIK.

38. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein encoded by clone 10 according to claim ~~18~~ binds, said composition comprising an effective amount of a protein encoded by clone 10 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein encoded by clone 10 with TRAF2 or any other molecule to which said protein encoded by clone 10 binds.

39. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a NIK protein, isoform, fragment, analog or derivative according to claim ~~19~~ or 20 binds, said composition comprising an effective amount of a NIK protein, isoform, fragment, analog or derivative thereof, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said NIK protein, isoform, fragment, analog or derivative thereof with TRAF2 or any other molecule to which said NIK protein, isoform, fragment, analog or derivative binds.

40. A method for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to ~~any one of claims 17-20~~ binds, said method comprising administering to a patient in need an effective amount of a protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to ~~any one of claims 17-20,~~ or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to ~~any one of claims 17-20~~ with TRAF2 or any other molecule to which said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one ~~of claims 17-20~~ binds.

41. A method according to claim 40 wherein said protein is encoded by clone 10.

42. A method according to claim 40, wherein said protein is NIK.

43. A method for screening of a ligand capable of binding to a ~~protein~~ according to ~~any one of claims 17-20~~ comprising contacting an affinity chromatography matrix to which said ~~protein~~ is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

44. A method for screening of a DNA sequence coding for a ligand capable of binding to a ~~protein~~ according to ~~any one of claims 17-20~~ comprising applying the yeast two-hybrid procedure in which a sequence encoding said ~~protein~~ is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

45. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by TRAF2 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of TRAF2 having the amino acid residues 222-501 of TRAF2;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

46. A method for identifying and producing a ligand capable of modulating the cellular activity modulated or mediated by a protein according to ~~any one of claims 17-20~~ comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

47. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by NIK comprising :

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a) screening for a ligand capable of binding to at least a portion of the NIK sequence ~~depicted in Fig. 6~~ of SEQ ID No:7;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

48. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by NIK, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by NIK;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

49. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by a ~~protein~~ polypeptide according to any one ~~of claims 17-20~~ claim 175, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by a ~~protein~~ polypeptide according to ~~any one of claims 17-20~~ claim 175;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

SUB H5 (cont'd)

add C3

add C4

ADD A3

add C10

add E2

add E5